Scientific Reports

https://doi.org/10.1038/s41598-025-34712-x

Article in Press

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Received: 26 June 2025

Accepted: 30 December 2025

Published online: 09 March 2026

Cite this article as: Lam V.P., Loi D.N., Bok G. *et al.* Effect of short-duration microwave treatments on flower development and secondary metabolite production in *Agastache rugosa*. *Sci Rep* (2025). https://doi.org/10.1038/s41598-025-34712-x

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Effect of Short-Duration Microwave Treatments on Flower Development and Secondary Metabolite Production in *Agastache rugosa*

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Abstract

This study investigated the effects of short-duration microwave (MW) exposure on growth, photosynthesis, antioxidant activity, and secondary metabolite accumulation in *Agastache rugosa* cultivated in a deep flow technique hydroponic system. Plants at 14 and 18 days after transplanting were exposed to MW radiation at 200 W for 5, 10, 15, 20, and 25 seconds, with untreated plants serving as the control. While most vegetative growth parameters were unaffected, MW exposure for 15–25 s significantly increased flower branch number by 9–15% and flower biomass by 9–24% compared with the control. These treatments also enhanced net photosynthetic rate (by up to 53%), chlorophyll a content (by 12%), and

total phenolics (by 43–85%) compared with the control. Antioxidant enzyme activities were markedly elevated, with SOD, POD, and CAT increasing by up to 66%, 49%, and 103%, respectively. MW exposure also promoted phytochemical accumulation: total flavonoids increased by 7–11%, and key bioactive compounds such as chlorogenic acid (up to 7.3-fold), tilianin (up to 53%), and rosmarinic acid (up to 42%) were significantly enhanced. These results indicate that short MW exposures of 15–25 s act as an effective elicitation strategy to improve flower development and phytopharmaceutical quality of *A. rugosa* under controlled cultivation conditions.

Keywords: acacetin; *Agastache rugosa*; antioxidant enzyme activity; chlorogenic acid; microwave elicitation; rosmarinic acid; tilianin

Introduction

Agastache rugosa is a perennial herb renowned for its diverse medicinal properties, including anti-inflammatory, antitumor, anti-atherogenic, antibacterial, antipyretic, anti-melanogenesis, antifungal, carminative, and antiviral [1]. It is widely used as both a culinary seasoning and a traditional medicinal herb in several Asian countries, including Korea, Vietnam, China, and Japan [2, 3]. *A. rugosa* possesses antioxidant [4], anticancer [5], anti-inflammatory [6], cardiovascular [7], and antimicrobial properties [8]. Tilianin, acacetin, and rosmarinic acid (RA), the primary bioactive compounds in *A. rugosa*, play a crucial role in its immunomodulatory effects while also exhibiting diverse pharmacological properties, including anti-inflammatory, antitumorigenic, antioxidative, neuroprotective, antidiabetic, cardioprotective, and anticancer effects [9-13]. Increasing concerns regarding the health-promoting qualities of food and the long-term viability of current farming systems emphasize the urgency of implementing sustainable strategies that enhance both the nutritional content and ecological value of *A. rugosa*.

Environmental stressors, such as heat stress, significantly influence plant growth and metabolism, often enhancing biosynthesis of secondary metabolites as a stress response [14]. Recent studies suggest that

controlled abiotic stressors, including MW radiation, can stimulate plant metabolic pathways and improve crop productivity and quality [15-17]. Plants are highly sensitive to external stimuli, with responses involving alterations in membrane potential, calcium ion fluxes, and reactive oxygen species (ROS)-mediated signaling cascades, that regulate downstream gene expression related to growth, stress tolerance, and secondary metabolite production [18-20]. Exploiting these responses through artificially induced electromagnetic radiation (ER), such as MW treatment, provides a novel strategy to enhance plant quality and bioactivity [21]. MW radiation, a form of electromagnetic fields (EF), has been reported to induce physiological and biochemical changes in plants [22, 23]. For example, MW exposure (4–5 h at 9.3 GHz) enhanced tomato seed germination, seedling vigor, and accumulation of such as polyphenols, flavonoids, chlorophyll, carotenoids, and proteins, while excessive exposure reduced growth and biochemical content [24]. Similarly, long-term exposure of duckweed to low-frequency ER (50 Hz) altered growth traits and induced point mutations in antioxidant genes (APx, GPx, Cat), particularly in intron regions, suggesting that chronic low-dose ER can influence plant growth and genetic stability [25]. EF synergized with SiO_2 nanoparticles to improve growth, antioxidant defense, and bioactive metabolite production in *Anthemis gilanica* plants [26]. ER exposure led to reductions in germination percentage, shoot length, root length, and leaf area in lettuce, while fresh and dry weight increased. Notably, flavonoid concentration showed a progressive rise with longer exposure durations [27]. Collectively, these findings demonstrate that MW radiation, when carefully controlled, can enhance plant productivity and nutritional value. However, despite growing evidence that magnetic fields can induce structural and physiological changes in plants, data on MW radiation effects, particularly regarding non-destructive changes and stress physiology, remain limited given that plants transmit electrical signals through interconnected pathways [18, 28]. Manipulating their electrical potential with external stimuli may alter signaling accuracy and physiological responses [29]. Therefore, investigating how different MW

exposure durations affect growth and secondary metabolite production is essential to optimize this technique as a sustainable cultivation strategy. To date, no study has examined these effects in *A. rugosa*.

This study investigated the impact of MW treatments on the growth and bioactive compound accumulation in *A. rugosa*. Specifically, the objectives are to (i) determine the optimal MW irradiation time to maximize flower development and (ii) evaluate its effects on the production of key secondary metabolites, such as RA, tilianin, and acacetin, as well as antioxidant and physiological stress responses. By examining the effects of controlled MW exposure, this research aims to provide valuable insights into the development of eco-friendly agricultural practices that enhance the quality and productivity of medicinal plants like *A. rugosa*.

Results

Plant growth parameters

Fig. 2 and Table 1 indicates that there were no significant differences in leaf length, leaf width, leaf number, leaf area (LA), stem length, stem fresh weight (FW), shoot and root FW, and root length among the MW treatment durations and the control ($p > 0.05$). However, the 15-, 20-, and 25-s MW treatments led to a statistically significant increase in the number of flower branches, by 9–15% compared with the control ($p = 0.002$), and flower FW, which rose by 19–24% ($p < 0.001$). By contrast, leaf FW was significantly reduced by 18–24% under 20- and 25-s MW treatments relative to the control ($p < 0.001$, Fig. 2 and Table 1). As shown in Fig. 2 and Table 2, flower dry weight (DW) was significantly enhanced under the 5-, 15-, 20-, and 25-s MW treatments (increased of 9–29%; $p < 0.001$), whereas leaf DW exhibited a significant decrease by 23–27% in the 20- and 25-s treatments, compared to the control **(**$p < 0.001$). Stem DW, root DW, shoot DW, specific LA, whole plant DW, and LA ratio showed no significant variation across the different MW treatment durations compared to the control ($p > 0.05$). Significant decreases by 26–33% in leaf weight ratio ($p < 0.001$) and increases in flower weight ratio (increases of 18–22%; $p =$

0.003) were observed under the 20- and 25-s MW treatments compared to the control (Fig. 2 and Table 2).

Chlorophyll and total carotenoid levels

The 25-s MW treatment showed the highest Chl a concentration (4.15 $mg \cdot g^{-1}$), which was 12% higher than the control (3.69 $mg \cdot g^{-1}$) ($p = 0.034$). By contrast, no significant difference in Chl b was observed among the MW duration treatments and the control (Fig. 3A and 3B). The total Chl (a+b) content was also maximized in the 25-s MW treatment, reaching 13% above the control ($p = 0.024$) (Fig. 3C). A significant increase in total carotenoid content was observed in the 10-s MW-treated plants (0.66 $mg \cdot g^{-1}$), which represented about 29% enhancement compared with the control (0.52 $mg \cdot g^{-1}$) ($p = 0.022$) (Fig. 3D).

Photosynthetic parameters

The net photosynthetic rate (A) was significantly enhanced in the 15-, 20-, and 25-s MW treatments compared with the control (4.05 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), reaching 5.31, 5.22, and 6.20 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, respectively—representing increases of 31%, 29%, and 53% over the control ($p < 0.001$) (Fig. 4A). Similarly, stomatal conductance (gs) exhibited a pronounced increase under the 25-s MW treatment (0.33 mol $H_2O \cdot m^{-2} \cdot s^{-1}$), which was 283% higher than the control (0.09 mol $H_2O \cdot m^{-2} \cdot s^{-1}$) ($p = 0.005$) (Fig. 4B). In contrast, intercellular CO_2 concentration (Ci) and transpiration rate (E) did not differ significantly among treatments ($p > 0.05$) (Fig. 4C and 4D), although a slight, non-significant upward trend was observed in the 25-s MW group.

Total flavonoid contents, total phenolic contents, and antioxidant activities

DPPH radical scavenging activity significantly increased in the 10-, 15-, 20-, and 25-s MW treatments, by 27%, 59%, 36%, and 30%, respectively, compared with the control ($p < 0.001$) (Fig. 5A). Exposure to MW for 15-, 20-, and 25-s significantly increased total flavonoid content by 7%, 10%, and 11%, respectively, compared with the control ($p < 0.001$) (Fig. 5B). Compared with the control, chlorogenic acid content markedly increased

under all MW treatments, showing up to a 7.3-fold rise at 10 s ($p < 0.001$). Caffeic acid levels also significantly increased in all treatments except at 15 s, reaching the highest value at 5 s (42% higher than the control, $p < 0.001$). Rutin content was significantly enhanced under the 10-, 15-, and 25-s treatments, with the 15-s treatment showing a 102% increase compared with the control ($p < 0.001$). In contrast, quercetin content decreased significantly under all MW treatments, whereas kaempferol content increased by 45% and 31% under the 15- and 25-s treatments, respectively ($p < 0.001$) (Table 7). Therefore, compared with the control (190.27 $mg \cdot g^{-1}$), all MW treatments significantly increased total phenolic content, reaching 271.21, 338.37, 323.98, 309.69, and 352.77 $mg \cdot g^{-1}$ DW at 5-, 10-, 15-, 20-, and 25-s, respectively—corresponding to 43%, 78%, 70%, 63%, and 85% increases ($p < 0.001$) (Fig. 5C). SOD activity increased with treatment duration, reaching its maximum under the 25-s treatment, which was 66% higher than the control ($p < 0.001$) (Fig. 5D). Compared to the control group, POD activity increased by 48.9%, 39.7%, 45.0%, and 36.0% following 10-, 15-, 20-, and 25-second MW treatments, respectively, indicating a substantial enhancement in enzymatic activity under these conditions ($p < 0.001$), whereas CAT activity was significantly enhanced by the 15-, 20-, and 25-second MW treatments, showing increases of 66.7%, 66.7%, and 103.3%, respectively, compared with the control ($p = 0.011$) (Fig. 5E and 5F).

Concentrations and contents of acacetin, tilianin, and RA

Among the various organs of *A. rugosa*, RA was predominantly found in the roots, whereas tilianin and acacetin were most abundant in the flowers, indicating significant organ-specific variation in compound accumulation. Significant increases in RA concentrations were observed in the flowers and stems after 15- and 25-s MW treatments, showing approximately 29% and 28% increases in flowers ($p < 0.001$) and 36% and 62% increases in stems ($p < 0.001$), respectively, compared to the control (Table 3). The 20-second treatment produced the highest RA concentration in leaves, representing a 24% increase relative to the control ($p < 0.001$), while RA accumulation in roots increased by 10% and 17% under the 20- and 25-s

treatments, respectively ($p < 0.001$). Tilianin concentrations increased by 18% and 26% in flowers following the 5- and 15-s treatments ($p = 0.010$), and by 31% and 32% in stems after the 10- and 20-s treatments, respectively ($p = 0.009$). Tilianin levels in leaves remained statistically unchanged across all treatments ($p > 0.05$). In contrast, roots exhibited substantial increases in tilianin concentration—approximately 21%, 25%, and 24% higher than the control after the 10-, 20-, and 25-s treatments ($p < 0.001$). MW exposure resulted in a slight reduction in acacetin concentrations in flowers (by approximately 10–40% under the 15- and 20-s treatments; $p < 0.001$) and a more pronounced decrease in stems (10–45% under the 5-, 10-, 15-, and 25-s treatments; $p < 0.001$) and leaves (6.7–14.5% under all treatments except 5-s; $p < 0.001$) compared to the control, while acacetin remained completely absent in the roots of *A. rugosa* (Table 3).

RA content reached its highest level in flowers under the 25-second treatment, showing an increase of approximately 33% compared to the control ($p < 0.001$), while the 20-second treatment resulted in the highest RA accumulation in roots (26% higher than the control) ($p < 0.001$) (Table 4). Stems and leaves also showed significant increases in RA content after the 15-, 20-, and 25-second treatments, corresponding to increases of 87%, 84%, and 122% in stems and 33%, 74%, 18% in leaves relative to untreated plants ($p < 0.001$). Tilianin content in flowers peaked under the 15-second treatment, showing an enhancement of about 56% compared to the control ($p < 0.001$). Stems and leaves exhibited significant increases under the 10-, 15-, 20-, and 25-second treatments, with rises of 73%, 64%, 97%, and 58% and 32%, 38%, 46%, and 39%, respectively ($p < 0.001$). Tilianin levels in roots were also significantly elevated, showing 24%, 68%, and 39%% increases under the 15-, 20-, and 25-second treatments relative to the control group ($p < 0.001$). Acacetin content in flowers reached its maximum under the 5-second treatment, increasing by approximately 21% compared to the control ($p < 0.001$). Stems showed notable increases of 19%, 15%, and 22% under the 10-, 15-, and 20-second treatments ($p <$

0.001), while leaves exhibited 18–21% higher acacetin content across all MW treatments ($p = 0.003$) (Table 4).

Whole plants exposed to a 20-s MW treatment exhibited significantly elevated levels of RA, with concentration and content increasing by 14% and 42%, respectively, compared with the control ($p < 0.001$) (Fig. 6A and 6D). All MW treatments markedly enhanced tilianin content by 24–53% ($p < 0.001$), whereas tilianin concentration increased significantly at 5-, 10-, 15-, and 25-s treatments by 16%, 14%, 20%, and 15% ($p = 0.005$), respectively, relative to untreated plants (Fig. 6B and 6E). In contrast, acacetin concentration gradually declined with increasing MW exposure, showing decreases of 12%, 31%, and 23% at 15-, 20-, and 25-s treatments ($p < 0.001$), respectively, compared to the control. However, its content increased by 13% and 16% at 10 and 15-s treatments, respectively, reaching the highest value at 15 s due to the influence of plant DW ($p = 0.001$) (Fig. 6C and 6F).

Concentrations and contents of acacetins 1, 2, and 3

Acacetin 1 concentrations in flowers and stems did not differ significantly between MW treatments and the control ($p > 0.05$) (Table 5). While acacetin 1 concentrations in leaves were significantly reduced from 21% to 38% under all MW treatments relative to the control ($p < 0.001$), The highest root concentration was observed under the 10-s treatment, showing a 75% increase compared with the control ($p < 0.001$). Acacetin 2 concentrations in flowers remained unaffected by MW treatments ($p > 0.05$); however, stems exhibited a significant increase 54%, 44%, 51%, and 68% under 10-, 15-, 20-, and 25-s exposures compared to the control group ($p < 0.001$). The 5-s MW treatment resulted in the lowest acacetin 2 concentration in leaves, showing an 18% decrease ($p < 0.001$), while simultaneously inducing the highest concentration in roots, with a 97% increase compared with the control ($p < 0.001$). MW treatments did not alter acacetin 3 concentrations in flowers and stems ($p > 0.05$), but significantly reduced its levels in leaves by 40% ($p < 0.001$) and, under the 25-s treatment, in roots by 26% ($p < 0.001$) (Table 5).

Although MW treatments did not significantly affect acacetin 1 content in flowers ($p > 0.05$), the 10- and 20-s exposures led to elevated levels in stems compared to the control group ($p < 0.001$) (Table 6). Leaf acacetin 1 content generally decreased under the 5-, 15-, and 25-s MW treatments by 23%, 8%, and 18% ($p < 0.001$), respectively, while no significant changes were observed at 10- and 20-s. In contrast, root acacetin 1 levels were significantly increased by 38% to 99% across all treatments compared with the control ($p < 0.001$). Acacetin 2 content increased significantly in flowers by 29% and 28% under the 20- and 25-s MW treatments, respectively, and in stems under the 10-, 15-, 20-, and 25-s treatments, showing 2.04-, 1.98-, 2.25-, and 2.31-fold increases compared with the control ($p < 0.001$). Acacetin 2 contents in leaves and roots, and acacetin 3 content in flowers, reached their highest levels under the 20-s treatment, increasing by 53% in leaves, 37% in roots, and 25% in flowers compared with the control ($p < 0.001$). The acacetin 3 content in stems was significantly higher under the 10, 15, 20, and 25 s treatments, showing increases of 57%, 41%, 67%, and 43% ($p < 0.001$), respectively, compared with the control. MW treatments reduced acacetin 3 content in leaves by 29%, 8%, and 21% under the 5 s, 15 s, and 25 s treatments, respectively, except at 10 s and 20 s ($p < 0.001$). In contrast, a significant increase in root acacetin 3 content was observed at 15 s and 20 s, with increases of 41% and 53% compared to the control ($p < 0.001$) (Table 6).

MW treatments caused a significant reduction in whole-plant acacetin 1 concentrations, except under the 10-s treatment. Acacetin 1 decreased by 17%, 16%, 15%, and 24% at 5-, 15-, 20-, and 25-s, respectively ($p < 0.001$) (Fig. 7A). In contrast, acacetin 2 levels showed no significant variation among treatments and the control ($p > 0.05$) (Fig. 7B). Notably, acacetin 3 decreased by 19% only under the 15-s treatment compared with the control ($p = 0.001$) (Fig. 7C). The contents of acacetin 1 and acacetin 3 in the whole plant reached their highest levels under the 20 s treatment, showing increases of 19% and 27%, respectively, compared with the control ($p = 0.001$) (Fig. 7D and 7F). The acacetin 2 content in the whole plant gradually increased by 24%, 30%, 42%, and 38% with increasing MW

exposure durations of 10 s, 15 s, 20 s, and 25 s, respectively ($p < 0.001$) (Fig. 7E).

Discussion

General vegetative parameters showed no significant differences among treatments, indicating that MW exposure did not negatively affect overall vegetative growth. Conversely, leaf FW and DW were significantly reduced under the 20- and 25-s treatments, potentially due to tissue dehydration or a shift in resource allocation toward flower production. Higher MW exposure may cause localized heating or cellular damage in leaves, leading to reduced water content (lower FW) and possibly degradation of cellular structures (lower DW). Stress signaling might lead to reallocation of resources away from vegetative organs (like leaves) towards reproductive structures (flowers) [30]. This shift was further supported by a significant decrease in the leaf weight ratio and a corresponding increase in the flower weight ratio in these treatments. MW treatment for 20 and 25 s significantly decreased the leaf weight ratio while increasing the flower weight ratio. This indicates that prolonged MW stress alters biomass allocation, favoring reproductive structures over vegetative growth [31]. Such a shift supports the concept of stress-adaptive reproductive prioritization, where plants allocate more resources to flowering as a survival strategy under adverse conditions [32]. Despite these changes, parameters such as stem DW, root DW, specific LA, whole plant DW, and LA ratio remained unaffected, implying that MW treatments selectively enhanced reproductive traits without impairing total plant biomass or structural development.

The increase in Chl a and total Chl content observed in the 25-s MW treatment group may be attributed to mild heat stress, which likely activated Chl biosynthesis pathways [33, 34]. This is consistent with earlier studies, in which tomato plants maintained at 31 °C were found to have 19.58% more chlorophyll and a darker green appearance than those grown at 26 °C [34]. Sweetpotato overexpressing IbOr sustains higher PSII efficiency and chlorophyll content under heat stress [35]. Short-duration

MW exposure at 14 and 18 days after transplanting, followed by a 20-day recovery period, likely acted as a mild stress that transiently increased membrane permeability and activated stress-related signaling pathways. These physiological responses may have promoted the uptake of key nutrients such as magnesium and nitrogen and stimulated chlorophyll biosynthetic enzymes, ultimately resulting in higher chlorophyll content observed at harvest [36, 37]. This mild stress likely promotes the activity of Chl biosynthetic enzymes without causing thermal damage. In contrast, Chl b levels remained unchanged across treatments, which may be due to its stability and its role as an accessory pigment that is less responsive to environmental changes compared to Chl a [38, 39]. Interestingly, the highest carotenoid content was recorded in the 10-s MW treatment, suggesting that shorter exposure may induce a protective response by increasing antioxidant compounds like carotenoids, which help mitigate oxidative stress [40]. These findings suggest that specific durations of MW treatment can selectively enhance pigment production, with longer exposure favoring chl accumulation and shorter exposure stimulating carotenoid biosynthesis.

The significant increase in photosynthetic rate (A) observed in the 15-, 20-, and 25-second MW treatment groups is likely due to mild heat stress induced by short-duration MW exposure at 200 W. This sub-lethal thermal stimulus may have activated photosynthetic enzymes, such as Rubisco, and enhanced chloroplast metabolism, thereby improving carbon fixation efficiency. Mild heat stress can also transiently stimulate electron transport and accelerate enzymatic reactions, increasing the rate of CO_2 assimilation into sugars without causing thermal damage [41, 42]. The increase in gs, particularly in the 25-s treatment, suggests greater stomatal opening, facilitating enhanced CO_2 influx and supporting the elevated photosynthetic rate. This response may be mediated by low levels of reactive oxygen species (ROS) and altered hormonal signals, such as abscisic acid and cytokinins, which modulate guard cell ion transport and turgor to regulate stomatal aperture [43]. The unchanged intercellular CO_2 concentration (Ci) under MW exposure indicates that the additional CO_2

entering the leaf via increased stomatal conductance was efficiently fixed in the Calvin cycle, reflecting enhanced carbon assimilation and photosynthetic efficiency [44]. Similarly, the stable transpiration rate (E) suggests that water loss was effectively regulated, likely through improved water-use efficiency and precise stomatal control mediated by ROS and hormonal signals [45]. Together, these responses demonstrate that MW-treated plants can optimize carbon fixation while maintaining water balance under mild heat stress, highlighting a coordinated adjustment between gas exchange and photosynthetic metabolism [46].

The observed increase in DPPH radical scavenging activity under all MW durations (10–25 s) suggests that MW exposure induces oxidative stress, which activates the plant's defense mechanisms and stimulates the production of antioxidant compounds [47]. Phenolic acids such as chlorogenic and caffeic acid were significantly elevated, likely due to enhanced activity of the phenylpropanoid pathway. Rutin content increased under certain treatments, while quercetin content consistently decreased, possibly due to its conversion into glycosylated forms or thermal degradation. Kaempferol content was elevated at 15- and 25-s exposures, indicating selective activation of flavonoid biosynthesis [47]. Moreover, total flavonoid and phenolic contents were significantly enhanced, particularly under longer exposures (15–25 s), reflecting the plant's adaptive response to moderate stress. Previous studies have similarly shown that MW treatment (40 s) elevates phenolics, flavonoids, and antioxidant potential in white and red sorghum, with diminishing effects under extended exposure [47]. Antioxidant enzyme activities, including SOD, POD, and CAT, were also significantly increased, indicating an active detoxification response to elevated ROS generated by MW stress. Consistent with earlier findings, MW treatment at 300 W for 75 s significantly enhanced the activities of SOD, CAT, POD, and APX enzymes in Tartary buckwheat sprouts, surpassing those in both the control and ungerminated seeds. This condition also maximized antioxidant capacity, as evidenced by increased reducing power and enhanced scavenging of DPPH, ABTS, O_2^-, and •OH radicals, indicating a notable improvement in

antioxidant potential [48]. Elevation of phenolic acids such as chlorogenic and caffeic acid likely reflects enhanced flux through the phenylpropanoid pathway, initiated by upregulation of phenylalanine ammonia-lyase (PAL) and subsequent enzymes such as cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL). ROS generated by MW act as signaling molecules that trigger transcriptional and post-translational activation of these enzymes, shifting carbon flow from primary metabolism toward hydroxycinnamic acids and flavonoid biosynthesis [49, 50]. Comparable responses have been reported in other species: MW or short heat exposures increased PAL activity and total phenolics in *Tartary buckwheat* [51]. and MW treatments also elevated phenolics, flavonoids, and antioxidant capacity in *Leptadenia pyrotechnica* [52]. These results suggest that short-term MW exposure can be an effective elicitor to enhance the antioxidant capacity and phytochemical content in plants [48].

MW exposure induced distinct organ-specific and compound-specific changes in secondary metabolite accumulation in *A. rugosa*. RA levels increased significantly across all organs, particularly in flowers (25 s), roots (20 s), and whole plants (20 s), likely due to the activation of the phenylpropanoid pathway triggered by mild abiotic stress. This stress, associated with moderate ROS production and heat, may enhance the activity of key enzymes such as PAL, C4H, and 4CL [53]. Tilianin, a glycosylated flavonoid, also increased in nearly all organs under MW exposure, especially in flowers (5 and 15 s), stems and leaves (10–25 s), and roots (15–25 s), suggesting that oxidative stress upregulates flavonoid biosynthetic enzymes like CHS, CHI, and FNS, while glycosylation may stabilize the compound [47]. In contrast, acacetin concentrations decreased in stems and leaves and were absent in roots, although its content peaked at 15 s due to DW effects, indicating its thermolability and possible conversion into glycosylated derivatives [54]. The decline in free acacetin may reflect its thermolability and/or rapid in vivo glycosylation to acacetin-glycosides by UDP-glycosyltransferases (UGTs), as acacetin commonly occurs in plants as glycosides and flavones are known to be sensitive to heat and oxidative conditions [55, 56]

MW exposure induced distinct changes in secondary metabolite accumulation in *A. rugosa*, depending on compound type and exposure duration. RA levels significantly increased, particularly at 20 s, likely due to the activation of the phenylpropanoid pathway under mild oxidative and thermal stress. This stress may have upregulated key enzymes such as PAL, C4H, and 4CL, enhancing RA biosynthesis [57]. Tilianin content increased in all MW treatments, while its concentration rose significantly at 5, 10, 15, and 25 s. As a glycosylated flavonoid, tilianin synthesis is promoted under moderate ROS levels, and glycosylation may stabilize the molecule against degradation [47]. In contrast, acacetin concentration decreased with longer MW exposure, but its content peaked at 15 s, likely due to increased DW, despite its thermolability and possible conversion to tilianin. Acacetin 1 and 3 also showed reduced concentrations but increased contents at 20 s, suggesting organ-specific redistribution and accumulation under stress. Meanwhile, acacetin 2 concentration remained unchanged, but its content gradually increased with longer MW exposure, indicating slow but steady biosynthesis and greater thermal stability [57]. These findings suggest that MW-induced stress can stimulate secondary metabolite biosynthesis, especially for more stable or glycosylated compounds, while thermolabile aglycones like acacetin may be partially degraded or transformed under prolonged exposure.

Conclusions

This study demonstrates that short-duration MW exposure, particularly for 15 to 25 s, can effectively enhance floral development and stimulate the accumulation of valuable bioactive compounds in *A. rugosa*, without adversely affecting overall plant growth. These treatments improved photosynthetic efficiency and antioxidant activity, and increased the levels of key phytochemicals such as chlorogenic acid, kaempferol, tilianin, and RA. The findings suggest that MW treatment is a promising abiotic elicitation strategy for improving the phytopharmaceutical quality of medicinal plants in controlled hydroponic systems. Further research is

needed to explore underlying molecular mechanisms and to optimize MW application for commercial-scale production.

Methods

Conditions for seedling development

A. rugosa seeds from Danong Co. Ltd. (Seoul, Korea) were surface-sterilized with 70% ethanol for 1 min, followed by 1% sodium hypochlorite (NaOCl) for 10 min, and then rinsed three times with sterile distilled water to remove residual disinfectant. The sterilized seeds were air-dried under aseptic conditions before sowing in a 40 × 60 cm Rockwool-filled tray. The seedlings were grown at 22.9/18°C with 70 ± 9% humidity, receiving 220 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD under a 16 h/8 h light/dark cycle, and supplied with Hoagland solution (EC 1.17 $dS \cdot m^{-1}$, pH 6.3) beginning 14 days after sowing (DAS). The use of *Agastache rugosa* plants for the research conducted in this work complies with relevant institutional, national, and international guidelines and legislation.

Treatments

Following transplantation into a DFT system (Fig. 1) at 28 DAS, plants were exposed to different durations of MW radiation using an MW oven (Model MW25B, 1000W, 2450 MHz) at two time points: 14 and 18 days after transplanting (DAT). These time points were chosen because, at 14 and 18 DAT, *A. rugosa* plants had reached an appropriate vegetative size and were physiologically stable with well-developed leaves and roots, ensuring they could tolerate short-term heating at 200 W. MW treatments were applied directly to the whole plants. Before exposure, the plants were briefly soaked in water for 2 min to maintain tissue hydration and prevent overheating. They were then exposed to MW radiation for 5, 10, 15, 20, or 25 seconds, depending on the treatment, and immediately returned to the hydroponic setup for recovery. A control group received no MW treatment. The MW experiment, arranged in a completely randomized design with two replicates, involved collecting plant samples 39 DAT for analysis. Six plants (n = 6) were used to measure growth parameters, whereas three

plants per replicate (n = 3) were analyzed for photosynthesis, pigments, DPPH activity, total flavonoids, antioxidants, and bioactive compounds

Plant growth parameters

Measurements included root, leaf, and stem lengths; leaf width and number, leaf area (LA); flower branch count; and the fresh and dry weights (DW) of leaves, shoots, stems, roots, and flowers. Biomass allocation was assessed using flower-to-DW and leaf-to-DW ratios, calculated by dividing flower DW, LA, or leaf DW by the total plant DW. These ratios indicate resource allocation to reproductive versus photosynthetic structures, which can reflect growth strategies, reproductive potential, and overall plant performance. Specific leaf area (SLA; total LA per unit leaf DW) was calculated to evaluate leaf thickness and light-capturing efficiency, traits linked to photosynthetic performance and stress adaptation [58, 59]. Each replicate included six plants (n = 6) for growth parameter evaluation.

Photosynthetic characteristics

Photosynthetic responses were measured on 3 plants per replicate with a LI-COR 6400 portable system (LI-COR Inc., Nebraska, USA), focusing on the third fully developed leaf from the shoot tip [60], and included net photosynthetic rate, stomatal conductance, CO_2 concentration, and transpiration rate. Measurements were automatically recorded in triplicate per treatment after setting the leaf chamber to 400 $\mu mol\ mol^{-1}$ CO_2, 300 $\mu mol\ m^{-2}\ s^{-1}$ PPFD, 25 °C, and 500 $cm^3\ s^{-1}$ airflow.

Photosynthetic pigments and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Samples (aerial parts) from each treatment group were immediately frozen in liquid nitrogen after harvest, stored at -70 °C in a deep freezer, and then transferred to a -50 °C dry freezer (TFD5503, IL Shinbiobase Co. Ltd., Daejeon, Korea) for four days for freeze-drying, and later ground, sieved, and analyzed for pigments and antioxidant activity via an Epoch microplate spectrophotometer (EMS) (EPOCH-SN, Agilent Technologies, USA). To analyze pigments and DPPH activity, 20 mg of dry powder (DP) was mixed with 2 mL of 90% methanol and centrifuged at 1308 × g for 20 minutes [61]. Absorbance was measured at 665.2, 652.4, and 470 nm to

quantify Chl a, Chl b, and carotenoids, respectively, and at 517 nm for DPPH activity, as described by Lam et al. (2024) and Rahman et al. (2015) [62, 63].

Individual Phenolic Compounds

Phenolic constituents in *A. rugosa* were quantified using a 1260 Infinity Series High-performance liquid chromatography (HPLC) system (1260 Infinity; Agilent Technologies Inc.) [64, 65]. An extract was prepared from 100 mg of DP using 2 mL of 80% MeOH, followed by 1 hour of sonication with intermittent vortexing. The extract was centrifuged (12,000 × g, 10 min), filtered (0.45 µm), and analyzed by HPLC on a Symmetry C18 column with 0.15% acetic acid in water (solvent A) and methanol (solvent B). The HPLC gradient program (98 minutes) efficiently separated five phenolic compounds—chlorogenic acid, caffeic acid, rutin, quercetin, and kaempferol—using a mobile phase B progression of: 5% (0–1 min), 15% (1–9 min), 20% (9–24 min), 30% (24–54 min), 45% (54–66 min), 56% (66–76 min), 60% (76–80 min), 80% (80–91 min), and 5% (91–98 min). HPLC was conducted using a C18 column maintained at 30 °C, with a mobile phase flow rate of 1.0 mL·min^{-1} and UV detection of phenolic acids at 280 nm. External standards for each phenolic compound were used to construct calibration curves. Standard solutions of chlorogenic acid, caffeic acid, rutin, quercetin, and kaempferol (Sigma-Aldrich, USA) were prepared in methanol at five concentration levels ranging from 100 to 500 µg·mL^{-1}. Calibration curves were generated by plotting peak area versus concentration, and all showed excellent linearity with R^2 values ≥ 0.9999. Retention times for major phenolic standards were as follows: chlorogenic acid (20.13 min), caffeic acid (27.59 min), rutin (61.75 min), quercetin (75.13 min), and kaempferol (82.08 min). Quantification of individual phenolic compounds in the samples was based on their corresponding calibration equations. Each sample was analyzed in triplicate with an injection volume of 20 µL.

Total flavonoid content and antioxidant activity

To determine the total flavonoid content, the method of Lam et al. (2024) [62] was employed using $AlCl_3$. A mixture was prepared containing 100 µL sample, 300 µL ethanol, 20 µL $AlCl_3$, 20 µL potassium acetate, and 600 µL distilled water. To quantify total flavonoids, samples were incubated for 40 minutes at 22 °C and their absorbance was read at 415 nm using an EMS. Quercetin served as the standard, and values were calculated in mg QE/g DW based on three replicates.

To determine the enzymatic activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), and POD (EC 1.11.1.7), a modified NBT assay was performed according to Lam et al. (2024) [62]. DP samples (20 mg) were homogenized in 2 mL of 50 mM phosphate buffer (pH 7.0), pre-treated with liquid nitrogen, and sonicated three times for 10 minutes each. Afterward, the homogenate was centrifuged at 1308 × g for 20 minutes at 4 °C. The supernatant was filtered through a 0.45 µm membrane and used for enzyme activity measurements.

SOD activity. SOD activity was measured by mixing 20 µL of sample with methionine, EDTA, NBT, PBS (pH 7.0), and riboflavin, followed by LED illumination (200 µmol m^{-2} s^{-1}, 8 min) and absorbance reading at 560 nm. SOD activity (U mg^{-1} DW) was defined as the amount of enzyme required to inhibit NBT photoreduction by 50%. SOD activity was quantified in U mg^{-1} DW and determined using the following formula:

$$\text{SOD (Unit mg}^{-1}\text{ DW)} = \frac{((\text{control - sample}) \times 100\% \times 200\ \mu\text{L})}{(\text{control} \times 50\% \times 20\ \mu\text{L} \times 0.2\ \text{mg})}$$

POD activity. POD activity was measured at 25 °C using PBS (pH 6.1), H_2O_2, and guaiacol, with the reaction started by adding 20 µL of sample. Absorbance at 470 nm was monitored, and activity was expressed as U mg^{-1} DW min^{-1}.

$$\text{POD (U mg}^{-1}\text{ DW min}^{-1}\text{)} = \frac{[(a^{\text{initial}} - a^{1\text{min}}) \times \text{enzyme liquid total volume (200 µL)}]}{[1\ (\text{min}) \times 20\ \mu\text{L} \times 0.2\text{mg sample quality}]}$$

Absorbance was recorded at 470 nm for 1 min using an extinction coefficient of 26.6 mM^{-1} cm^{-1}.

CAT activity. CAT activity was determined following Aebi's (1984) method, [66], The assay mixture contained 193.6 µL of PBS (pH 7.0) and 3.4 µL of 3% H_2O_2. The reaction was initiated by adding 3 µL of the sample extract. A blank mixture without enzyme extract was used as a reference and equilibrated in the spectrophotometer for 4–5 minutes. Absorbance was measured at 240 nm for 3 min using an EMS. CAT activity was calculated as the enzyme amount decomposing 1 µM of H_2O_2 per minute and expressed as µmol mg^{-1} DW min^{-1}.

$$\text{CAT (µmol ml}^{-1}\text{ min}^{-1}\text{)} = \frac{\text{(A240/min) } \times \text{ total volume } \times 1000}{43.6 \times \text{enzyme volume}}$$

$$\text{CAT (µmol mg}^{-1}\text{ DW min}^{-1}\text{)} = \frac{\text{µmol min}^{-1}\text{ ml}^{-1}}{\text{enzyme (mg ml}^{-1}\text{)}}$$

Absorbance was recorded at 240 nm for 1 min using an extinction coefficient of 43.6 M^{-1} cm^{-1}.

Concentrations and contents of acacetin, tilianin, three acacetin glycosides, and RA

Samples (roots, stems, leaves, and flowers) from each treatment group were immediately frozen in liquid nitrogen after harvest, stored at -70 °C in a deep freezer, and then transferred to a -50 °C dry freezer (TFD5503, IL Shinbiobase Co. Ltd., Daejeon, Korea) for four days for freeze-drying. After grinding with a mortar and pestle and filtering through a mesh, levels of RA, tilianin, acacetin, and its glycosides acacetin 7-O-(2"-O-acetyl)β-D-glucopyranoside (acacetin 1), acacetin 7-O-(6"-O-malonyl)β-D-glucopyranoside (acacetin 2), and acacetin 7-O-(2"-O-acetyl-6"-malonyl)β-D-glucopyranoside (acacetin 3) were analyzed following the method of Lam et al. (2024) [62]. 200 mg of DP was extracted in 10 mL of pure methanol, followed by 30 minutes of sonication, centrifugation at 1358 × g for 20 minutes, and filtration through a 0.45-µm filter. HPLC was conducted with a mobile phase of acetonitrile (B) and 0.1% formic acid in water (A), using a gradient of 20% B (0–5 min), 50% B (5–20 min), and 100% B (20–22 min). A 10 µL sample was injected at a flow rate of 0.8 mL·min^{-1}, and detection at 330 nm was calibrated using Sigma-Aldrich standards. RA, tilianin, acacetin, and acacetins 1, 2, and 3 showed distinct retention times

of 11.655, 12.542, 19.659, 13.131, 14.485, and 15.351 minutes. Standard compounds of RA, tilianin, acacetin, and acacetin 1, acacetin 2, and acacetin 3 were purchased from Sigma-Aldrich Corporation (Sigma-Aldrich, Co., Ltd., Seoul, Korea). The calibration curves for RA, tilianin, acacetin, and its glycosides showed excellent linearity (R^2 = 0.9970–1.0000) across 0.1–100 μg mL^{-1}, with LOD and LOQ values of 0.06–0.90 and 0.20–2.73 μg mL^{-1}, confirming high analytical sensitivity and reliability. Concentrations of these compounds in the whole plant (mg·g^{-1} DW) were determined by using Equation (1).

BCx in the whole plant (mg□g^{-1} DW) = □ (amount of BCx in each part × % DW of each plant organ per DW of the entire plant). (1)

BC: Bioactive compound

x: Tilianin, acacetin 1-3, or RA

By multiplying the concentration of each compound by the plant's total DW, the total content of BC per plant (mg/plant DW) was obtained.

Statistical analysis

A total of six plants (n = 6) were evaluated for growth traits, whereas photosynthesis, pigment composition, DPPH scavenging activity, total flavonoids, antioxidant capacity, and BC were analyzed using three plants per replicate (n = 3). Data from the MW experiment, arranged in a completely randomized design with two replicates, were statistically evaluated using one-way ANOVA and Tukey's multiple comparison test in SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

Authors' contributions: V.P.L.: Experimental design, experimental conducting, investigation, data collection and analysis, writing – original manuscript, and writing – review and editing. **D.N. L.:** Experimental conducting, investigation and preparation of the manuscript, and writing – review and editing. **G.B:** Investigation, data collection and analysis, writing – original manuscript, and writing – review and editing. **J.S.P.:** Project administration, supervision, experimental design, data analysis, writing – original manuscript, and writing – review and editing.

Data availability: The raw data of this study is available by the corresponding author up on request.

Competing interests: The authors have no conflicts of interest.

Funding: This work was supported by Korea Institute of Energy Technology Evaluation and Planning (KETEP) grant funded by the Korea government (MOTIE) (20212020800050, Development and demonstration of rooftop greenhouse-building integrated system using distributed polygeneration).

Acknowledgements: Not applicable.

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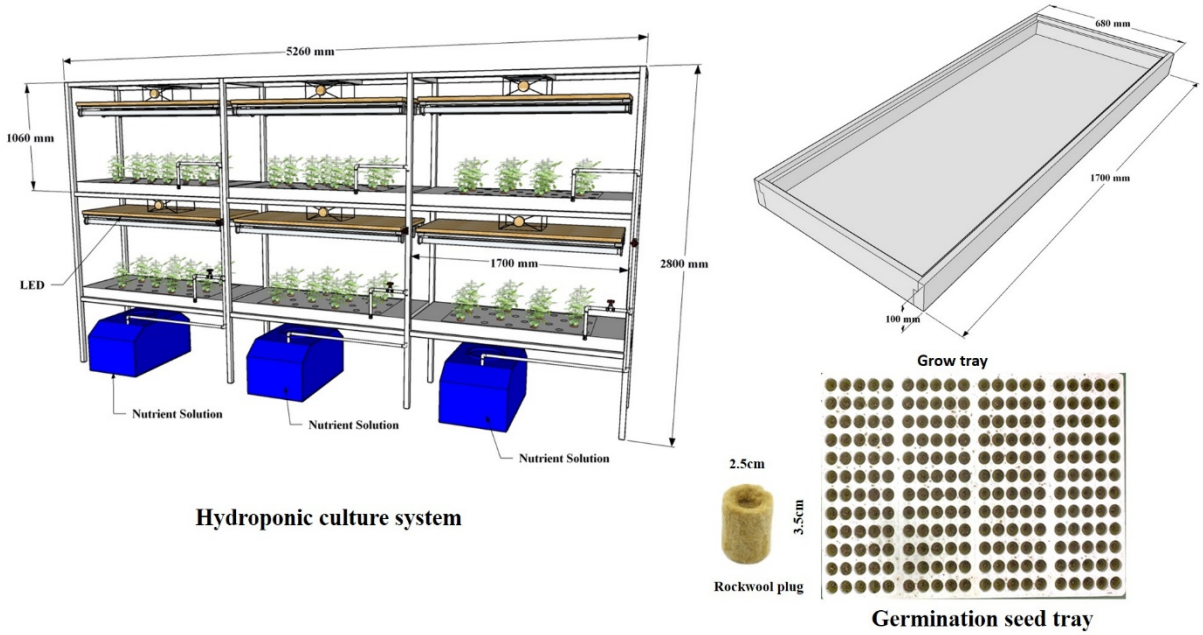

Fig 1. Germination seed tray, and a deep flow technique system in a plant factory.

Fig. 2. *A. rugosa* growth after 39 days of transplantation under various microwave treatment durations (0, 5, 10, 15, 20, and 25 s).

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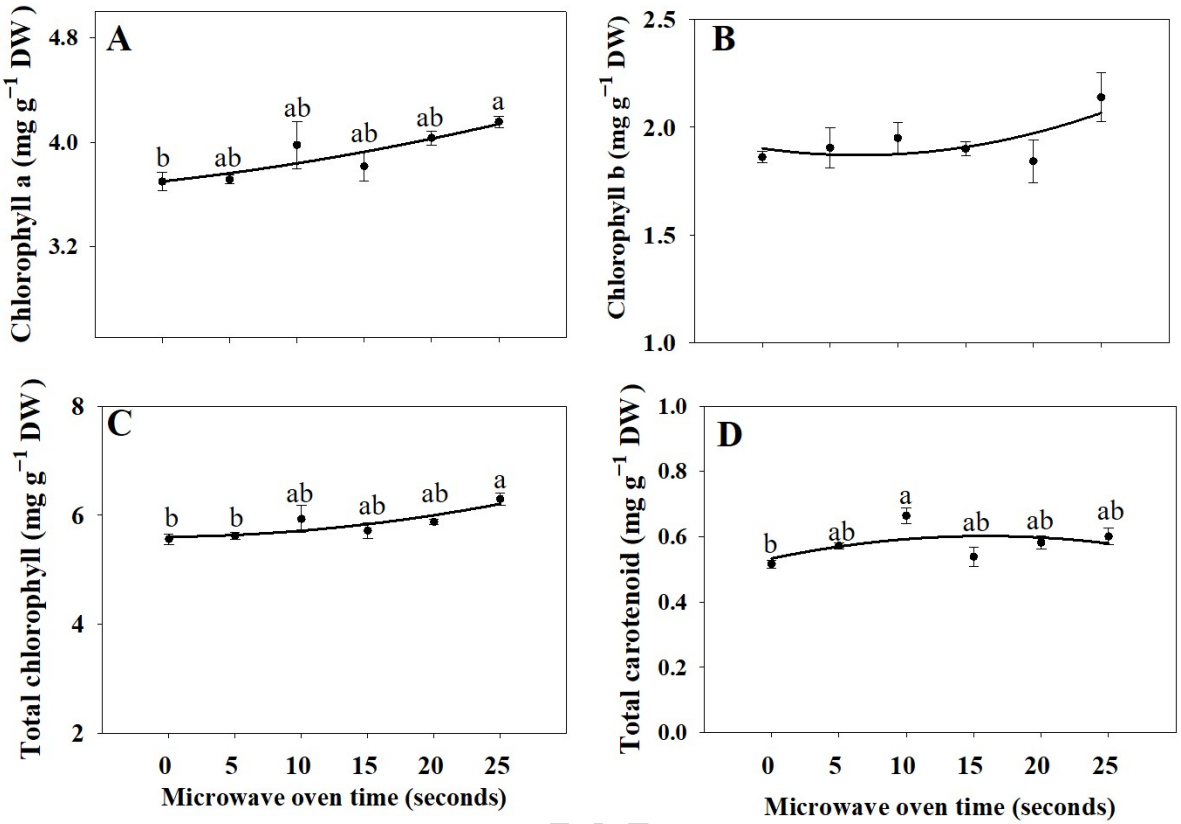


866

867 **Fig. 3.** Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and total
868 carotenoid (D) of *A. rugosa* under different microwave time treatments (0,
869 5, 10, 15, 20, and 25 s). Values are means ± SE (n = 3). Different letters
870 denote statistically significant differences ($p \leq 0.05$) based on ANOVA and
871 Tukey's post hoc test.

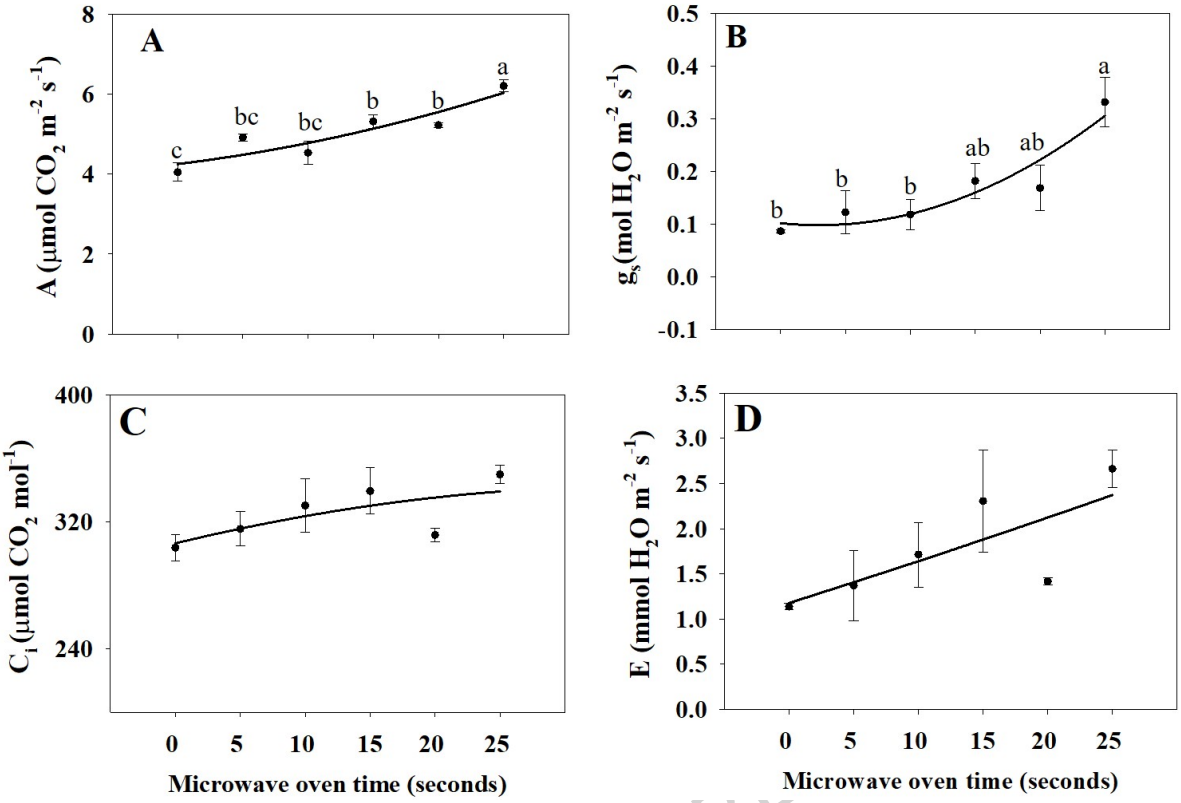


Fig. 4. Net photosynthetic rate (A; **A**), stomatal conductance (gs; **B**), intercellular CO_2 concentration (Ci; **C**), and transpiration rate (E; **D**) of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s). Data represent the mean ± standard error (SE) of three replicates (n = 3). Different letters indicate significant differences at $p \leq 0.05$, determined by one-way ANOVA followed by Tukey's multiple range test.

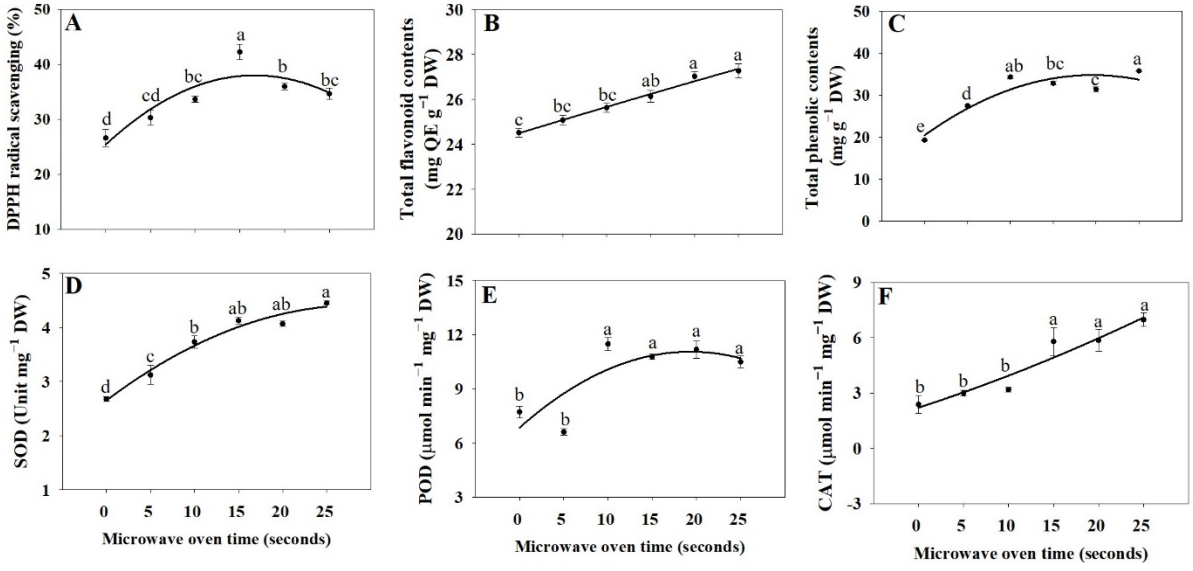


Fig 5. DPPH radical scavenging activity (A), total flavonoid levels (B), total phenolic contents (C), superoxide dismutase (SOD) levels (D), peroxidase (POD) levels (E), and catalase (CAT) levels (F) of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s). Values are means ± SE (n = 3). Different letters denote statistically significant differences ($p \leq 0.05$) based on ANOVA and Tukey's post hoc test.

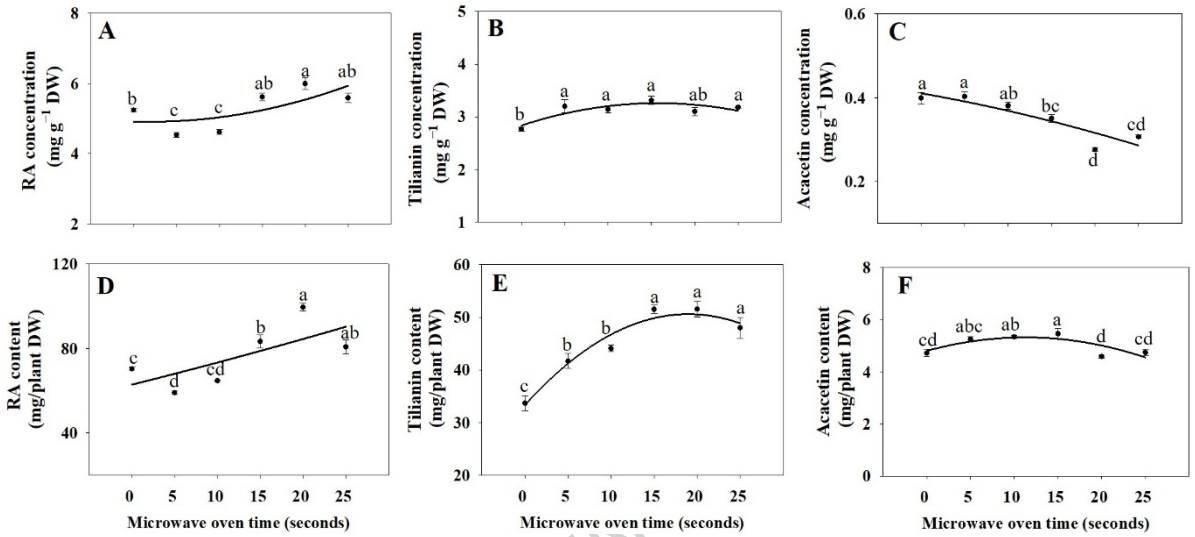
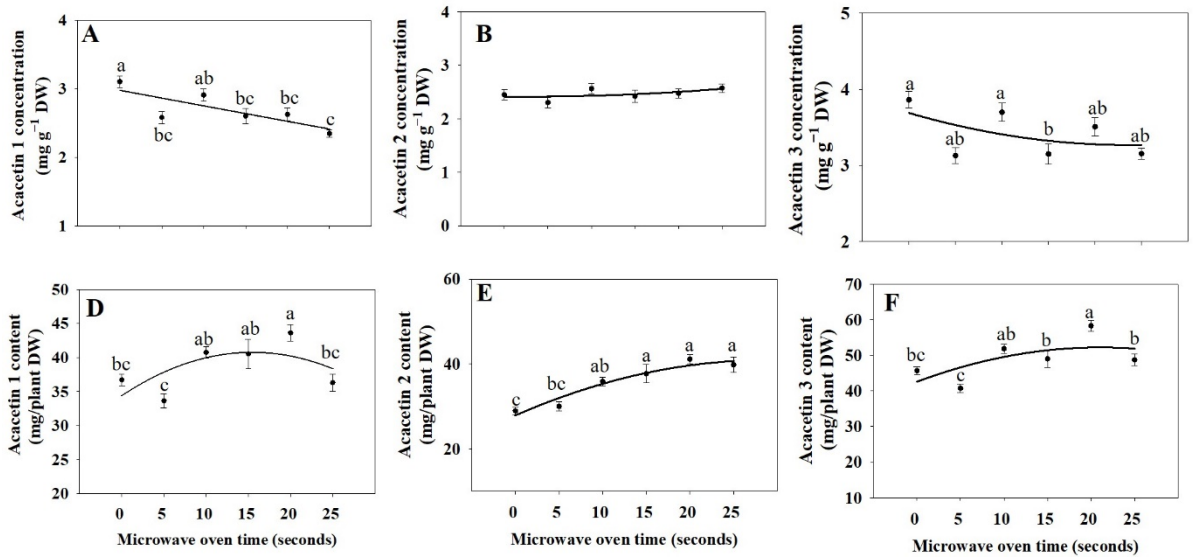


Fig. 6. Rosmarinic acid (RA) concentration (A) and content (D), tilianin concentration (B) and content (E), and acacetin concentration (C) and content (F) in *A. rugosa* whole plant under different microwave time treatments (0, 5, 10, 15, 20, and 25 s). Each value indicates the mean ± SE of three samples (n = 3). Different letters represent the significant differences at $p \leq 0.05$, as assessed using ANOVA, followed by Tukey's multiple range test.



894

895 **Fig. 7.** Acacetin 7-O-2"-O-acetyl)β-D-glucopyranoside (acacetin 1; A and D),
896 acacetin 7-O-(6"-O-malonyl)β-D-glucopyranoside (acacetin 2; B and E), and
897 acacetin 7-O-(2"-O-acetyl-6"-malonyl)β-D-glucopyranoside (acacetin 3; C
898 and F), concentrations and contents in *A. rugosa* whole plant under
899 different microwave time treatments (0, 5, 10, 15, 20, and 25 s). Each value
900 indicates the mean ± SE of three samples (n = 3). Different letters
901 represent the significant differences at $p \leq 0.05$, as assessed using ANOVA,
902 followed by Tukey's multiple range test.

Table 1. Growth characteristics of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	Leaf length (cm)	Leaf width (cm)	Flower branch number	Number of leaves (leaves)	Leaf area (cm^2)	Stem length (cm)	Flower FW (gram)	Leaf FW (gram)	Stem FW (gram)	Shoot FW (gram)	Root FW (gram)	Root length (cm)
0	9.03a	7.90ab	19.50b	154.67ab	1810.26ab	48.42a	12.71c	27.66a	20.22a	60.58a	25.02a	70.67ab
5	9.55a	7.10b	19.17b	139.17b	1389.11b	51.25a	12.725c	26.33a	21.87a	60.92a	26.48a	70.90ab
10	9.17a	7.23b	19.17b	195.50a	1950.88a	49.95a	12.87bc	24.78ab	23.96a	61.63a	23.99a	64.48b
15	9.43a	7.58ab	21.33a	174.33ab	1703.57ab	49.23a	15.79a	25.47ab	23.82a	65.09a	31.16a	74.22a
20	9.88a	8.10a	22.00a	193.50a	1733.39ab	50.48a	15.08ab	20.99c	24.62a	60.69a	32.43a	72.20ab
25	9.35a	7.70ab	21.67a	166.67ab	1766.86ab	50.77a	15.59a	22.28bc	25.15a	63.02a	25.21a	68.68ab
Significance[z]	NS	*	**	*	*	NS	***	***	NS	NS	NS	*

[w]MW time; Values represent the mean of six samples (n = 6). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. FW: fresh weight

Table 2. Growth characteristics of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	Flower DW (gram)	Leaves DW (gram)	Stem DW (gram)	Root DW (gram)	Shoot DW (gram)	Specific leaf area (SLA) (cm^2 /g)	Whole plant DW (gram)	Leaf area ratio (Cm^2/g)	Leaf weight ratio	Flower weight ratio
0	2.79c	5.51a	3.79ab	1.99ab	11.73a	331.24ab	13.72a	124.51ab	0.40a	0.20b
5	3.16b	5.14a	3.43b	2.01ab	12.10a	270.94b	14.112a	98.55b	0.36ab	0.22ab
10	3.04bc	4.93ab	4.13ab	1.87b	12.10a	394.29a	13.972a	138.92a	0.35ab	0.22ab
15	3.55a	5.12a	4.15ab	2.36ab	12.81a	334.97ab	15.18a	112.59ab	0.34ab	0.23ab
20	3.59a	3.98c	4.63a	2.65a	12.21a	448.49a	14.86a	117.25ab	0.27b	0.24a
25	3.51a	4.22bc	4.38a	2.02ab	12.10a	423.29a	14.12a	133.52a	0.29b	0.25a
Significance[z]	***	***	*	*	NS	*	NS	**	**	**

[w]MW time; Values represent the mean of six samples (n = 6). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. DW: dry weight

Table 3. Rosmarinic acid (RA), tilianin, and acacetin concentrations (mg·g^{-1} DW) in the flowers, stems, leaves, and roots of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	RA concentration in plant organs (mg·g^{-1} DW)				Tilianin concentration in plant organs (mg·g^{-1} DW)				Acacetin concentration in plant organs (mg·g^{-1} DW)			
	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots
0	4.13bc	0.80c	4.83b	15.11cd	7.39b	0.91b	2.34a	0.35b	0.95ab	0.39a	0.21a	ND
5	3.71c	0.79c	3.86cd	14.32d	8.75a	0.88b	2.36a	0.17c	0.98a	0.31b	0.22a	ND
10	4.47abc	0.52d	3.71d	15.93bc	8.68ab	1.19a	2.52a	0.42a	0.98a	0.35ab	0.19b	ND
15	5.33a	1.09ab	4.98b	15.31cd	9.34a	1.08ab	2.50a	0.35b	0.85bc	0.33b	0.19b	ND
20	4.76ab	0.98bc	5.99a	16.61b	8.59ab	1.20a	2.42a	0.44a	0.56d	0.32b	0.18c	ND
25	5.31a	1.29a	4.31c	17.72a	8.51ab	1.05ab	2.46a	0.44a	0.77c	0.21c	0.19bc	ND
Significance[z]	***	***	***	***	**	**	NS	***	***	***	***	ND

[w]MW time; Values represent the mean of three samples (n = 3). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. ND: not detected; RA: rosmarinic acid; DW: dry weight.

Table 4. RA, tilianin, and acacetin contents (mg/plant organs DW) in the flowers, stems, leaves, and roots of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	RA content in plant organs (mg/plant organs DW)				Tilianin content in plant organs (mg/plant organs DW)				Acacetin content in plant organs (mg/plant organs DW)			
	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots
0	14.44bc	3.05b	16.17c	34.87bc	21.03c	3.44b	7.83b	0.69c	2.59b	1.49bc	0.71b	ND
5	11.86c	3.26b	14.52c	28.39d	27.96ab	3.63b	8.87b	0.33d	3.14a	1.28cd	0.84a	ND
10	13.21c	2.62b	15.25c	30.72cd	25.66bc	5.98a	10.38a	0.82bc	2.89ab	1.77a	0.82a	ND
15	14.62bc	5.72a	21.59b	37.91b	32.89a	5.65a	10.83a	0.86b	3.01ab	1.72ab	0.85a	ND
20	17.21ab	5.59a	28.24a	43.90a	31.06ab	6.78a	11.41a	1.16a	2.03c	1.82a	0.86a	ND
25	19.26a	6.78a	19.09b	33.25bcd	29.57ab	5.46a	10.87a	0.96b	2.79ab	1.12d	0.84a	ND
Significance[z]	***	***	***	***	***	***	***	***	***	***	**	ND

[w]MW time; Values represent the mean of three samples (n = 3). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. ND: not detected; RA: rosmarinic acid; DW: dry weight.

Table 5. Acacetin 7-O-2"-O-acetyl)β-D-glucopyranoside (acacetin 1), acacetin 7-O-(6"-O-malonyl)β-D-glucopyranoside (acacetin 2), and acacetin 7-O-(2"-O-acetyl-6"-malonyl)β-D-glucopyranoside (acacetin 3) concentrations (mg.g^{-1} DW) in the flowers, stems, leaves, and roots of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	Acacetin 1 concentration in plant organs (mg·g^{-1} DW)				Acacetin 2 concentration in plant organs (mg·g^{-1} DW)				Acacetin 3 concentration in plant organs (mg·g^{-1} DW)			
	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots
0	6.05a	1.42ab	4.07a	0.03d	7.84a	0.67b	1.38a	0.06d	8.47ab	1.87ab	3.93a	0.07a
5	5.43a	1.27ab	2.78cd	0.05ab	7.02a	0.72b	1.13b	0.13a	7.64ab	1.65b	2.47d	0.08a
10	6.07a	1.58a	3.21b	0.06a	8.13a	1.03a	1.48a	0.09c	8.99a	2.21a	3.21b	0.07a
15	5.48a	1.36ab	2.89c	0.05ab	7.12a	0.96a	1.55a	0.09c	7.39b	1.91ab	2.79c	0.08a
20	5.37a	1.56a	2.97bc	0.05ab	7.65a	1.01a	1.49a	0.11b	9.01a	2.10ab	3.15b	0.08a
25	5.04a	1.11b	2.51d	0.04c	7.59a	1.12a	1.37a	0.09c	7.71ab	1.94ab	2.35d	0.05b
Significance[z]	NS	**	***	***	NS	***	***	***	**	*	***	***

[w]MW time; Values represent the mean of three samples (n = 3). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. DW: dry weight.

Table 6. Acacetins 1, 2, and 3 contents (mg/plant organs DW) in the flowers, stems, leaves, and roots of *A. rugosa* under different microwave time treatments (0, 5, 10, 15, 20, and 25 s).

[w]MW oven time treatments (seconds)	Acacetin 1 content in plant organs (mg/plant organs DW)				Acacetin 2 content in plant organs (mg/plant organs DW)				Acacetin 3 content in plant organs (mg/plant organs DW)			
	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots	Flowers	Stems	Leaves	Roots
0	16.46	5.38bc	13.60a	0.07c	21.32b	2.54b	4.62c	0.12e	24.52b	7.08b	13.14ab	0.13bc
5	17.35	5.20c	10.48c	0.09b	22.43ab	2.94b	4.26c	0.25b	24.42b	6.78b	9.284d	0.15b
10	17.98	7.94a	13.20a	0.11ab	24.06ab	5.17a	6.07b	0.17d	26.63ab	11.10a	13.184ab	0.13bc
15	19.37	7.15ab	12.49ab	0.13a	25.18ab	5.03a	6.73ab	0.23bc	26.11ab	10.03a	12.084bc	0.19a
20	19.41	8.81a	13.98a	0.12a	27.62a	5.72a	7.06a	0.29a	30.59a	11.87a	14.84a	0.21a
25	18.22	5.79bc	11.08bc	0.09b	27.47a	5.86a	6.07b	0.21c	27.88ab	10.16a	10.394cd	0.11c
Significance[z]	NS	***	***	***	*	***	***	***	*	***	***	***

[w]MW time; Values represent the mean of three samples (n = 3). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments.

NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$. DW: dry weight.

Table 7. Phenolic compounds in *A. rugosa* harvested at 39 days after transplanting under different microwave oven time treatments (0-, 5-, 10-, 15-, 20-, and 25-second).

[w]MW oven time treatments (seconds)	Phenolic compounds in *A. rugosa* (mg·g^{-1} DW)				
	Chlorogenic acid (mg·g^{-1} DW)	Caffeic acid (mg·g^{-1} DW)	Rutin (mg·g^{-1} DW)	Quercetin (mg·g^{-1} DW)	Kaempferol (mg·g^{-1} DW)
0	2.41 ± 0.074d	2.30 ± 0.125c	0.57 ± 0.014c	6.02 ± 0.049a	7.98 ± 0.219b
5	9.84 ± 0.083c	3.27 ± 0.021a	0.79 ± 0.013bc	5.37 ± 0.041b	8.22 ± 0.372b
10	17.59 ± 0.323a	3.13 ± 0.033ab	0.82 ± 0.094b	4.66 ± 0.076c	8.08 ± 0.139b
15	12.41 ± 0.496b	2.41 ± 0.062c	1.15 ± 0.024a	5.31 ± 0.115b	11.54 ± 0.131a
20	13.55 ± 0.202b	2.98 ± 0.027b	0.75 ± 0.073bc	5.29 ± 0.212b	8.84 ± 0.364b
25	16.89 ± 0.451a	2.99 ± 0.034 ab	0.82 ± 0.020b	4.61 ± 0.192c	10.42 ± 0.482a
Significance[z]	***	***	***	***	***

[w]MW time; Values represent the mean ±SE (standard error) value of three samples (n = 3). Data were analyzed using one-way ANOVA, and mean comparisons were performed using Tukey's multiple range test at $p \leq 0.05$. Different letters within a column indicate significant differences among treatments. NS: not significant ($p > 0.05$); [z]significant at *$p \leq 0.05$, **$p \leq 0.01$,

and ***$p \leq 0.001$.